(d) *Procedure.* Pour the developing solvent into the glass trough on the bottom of the tank and onto the paper lining the walls of the tank. Cover and seal the tank. Allow it to equilibrate for 1 hour. Prepare a plate as follows: On a line 2 centimeters from the base of the silica gel plate, and at intervals of 2 centimeters, spot 10 microliters each of the standard solution and the sample solution. After all spots are thoroughly dry, place the silica gel plate directly into the glass trough. Cover and seal the tank. Allow the solvent front to travel about 15 centimeters from the starting line. Remove the plate from the tank and air dry. Expose the plate to iodine vapors for 40 minutes. Immediately circumscribe all spots using a suitable marker.

(e) *Evaluation.* Measure the distance the solvent front traveled from the starting line and the distance the spots are from the starting line. Calculate the R_f value by dividing the latter by the former. The sample and standard should have spots of corresponding R_f values and intensity.

[46 FR 61070, Dec. 15, 1981, as amended at 49 FR 2242, Jan. 19, 1984]

§ 436.334 High-pressure liquid chromatographic assay for piperacillin.

(a) *Equipment.* A high-pressure liquid chromatograph equipped with:

(1) A low dead volume cell 8 to 20 microliters;

(2) A light path length of 1 centimeter;

(3) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;

(4) A suitable recorder of at least 25.4-centimeter deflection;

(5) A suitable integrator;

(6) A 25-centimeter column having an inside diameter of 4.6 millimeters and packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles, 5 to 10 micrometers in diameter (United States Pharmacopeia XX).

(b) *Reagents.* (1) 0.2*M* monobasic sodium phosphate: Dissolve 27.60 grams of monobasic sodium phosphate with sufficient water to make 1,000 milliliters.

(2) 10 percent tetrabutylammonium hydroxide in water.

(3) Ampicillin-piperacillin solution: Dissolve and dilute 25 milligrams of ampicillin and 5 milligrams of piperacillin monohydrate with sufficient mobile phase to obtain 100 milliliters, and mix.

(c) *Mobile phase.* Methanol:water:0.2*M* monobasic sodium phosphate:10 percent tetrabutylammonium hydroxide (450:447:100:3) adjusted to pH 5.5±0.02 with phosphoric acid. The concentration of reagents may be varied to obtain acceptable operation of the system. De-gas the mobile phase just prior to its introduction into the chromatograph pumping system.

(d) *Preparation of working standard and sample solutions*—(1) *Working standard solution.* Place approximately 20 milligrams of the working standard, accurately weighed, into a 50-milliliter volumetric flask. Add 25 to 30 milliliters of mobile phase. Shake until dissolved. Dilute to volume with mobile phase.

(2) *Sample solution*—(i) *Micrograms per milligram.* Place approximately 20 milligrams of the sample, accurately weighed, into a 50-milliliter volumetric flask. Add 25 to 30 milliliters of mobile phase. Shake until dissolved. Dilute to volume with mobile phase.

(ii) *Milligrams per vial.* Reconstitute as directed in the labeling. Withdraw the total contents and dilute with mobile phase to a concentration of 0.4 milligram of piperacillin per milliliter.

(e) *Procedure.* Use the equipment, reagents, mobile phase, and working standard and sample solutions described in paragraphs (a), (b), (c), and (d) of this section and proceed as directed in paragraph (e) of this section.

(1) *Systems suitability test.* Chromatograph three replicate samples of ampicillin-piperacillin solution as directed in paragraph (e)(2) of this section. Allow an elution time sufficient to obtain satisfactory separation of expected components after each injection. Record the peak responses and calculate the resolution factor as described for system suitability tests in the United States Pharmacopeia XX General Chapter 621 for gas chromatography. The resolution factor between ampicillin and piperacillin is not

less than 15. If the resolution factor does not meet this limit, adjustments must be made to the system to obtain satisfactory operation before proceeding as described in paragraph (e)(2) of this section.

(2) *Determination of the chromatogram.* Operate the high-pressure liquid chromatograph at ambient temperature at a flow rate of one milliliter per minute. Use a detector sensitivity setting that gives a peak height for the reference standard that is at least 50 percent of scale. Purge the column with mobile phase until a steady baseline is established. Inject 10 microliters of the working standard solution into the chromatograph. Allow an elution time sufficient to obtain separation of the expected components. After separation of the working standard solution has been completed, inject 10 microliters of the sample solution into the chromatograph and repeat the procedure described for the working standard solution.

(f) *Calculations*—(1) Calculate the piperacillin content in micrograms per milligram as follows:

$$\text{Micrograms of piperacillin per milligram of sample} = \frac{A \times \text{Weight of standard in milligrams} \times \text{Potency of working standard in micrograms per milligram}}{B \times \text{Weight of sample in milligrams}}$$

where:
A=Area of the sample peak (at a retention time equal to that observed for the standard);
B=Area of the standard peak.

(2) Calculate the piperacillin content in grams per vial as follows:

$$\text{Grams of piperacillin per vial} = \frac{A \times \text{Milligrams of standard in milligrams per milliliter} \times \text{Potency of working standard in micrograms per milligram} \times d}{B \times 1{,}000 \times 1{,}000}$$

where:
A=Area of the sample peak (at a retention time equal to that observed for the standard);
B=Area of the standard peak;
d=Dilution factor.

[47 FR 15768, Apr. 13, 1982; 47 FR 33493, Aug. 3, 1982]

§ 436.335 High-pressure liquid chromatographic assay for chloramphenicol palmitate.

(a) *Equipment.* A suitable high-pressure liquid chromatograph equipped with:

(1) A low dead volume cell 8 to 20 microliters;

(2) A light path of 1 centimeter;

(3) A suitable ultraviolet detection system operating at a wavelength of 280 nanometers;

(4) A suitable recorder of at least 25.4-centimeter deflection;

(5) A suitable integrator; and

(6) A 30-centimeter column having an inside diameter of 4.0 millimeters and packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles, 5 to 10 micrometers in diameter, U.S.P. XX.

(b) *Mobile phase.* Mix methanol:water:glacial acetic acid (170:30:1). Degas the mobile phase just prior to its introduction into the chromatograph pumping system.

(c) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 2.0 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the reference standard that is at least 50 percent of scale. The minimum between peaks must be no more than 2 millimeters above the initial baseline.

(d) *Preparation of sample and working standard solutions.* Accurately weigh approximately 65 milligrams of sample